Oxidative damage-related genes *AKR1C3* and *OGG1* modulate risks for lung cancer due to exposure to PAH-rich coal combustion emissions

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Lung cancer rates among men and particularly among women, almost all of whom are non-smokers, in Xuan Wei County, China are among the highest in China and have been causally associated with exposure to indoor smoky coal emissions that contain very high levels of polycyclic aromatic hydrocarbons (PAHs). As such, this population provides a unique opportunity to study the pathogenesis of PAH-induced lung cancer that is not substantially influenced by the large number of other carcinogenic constituents of tobacco smoke. Aldo-keto reductases (AKRs) activate PAH dihydrodiols to yield their corresponding reactive and redox-active *o*-quinones, which can then generate reactive oxygen species that cause oxidative DNA damage. We therefore examined the association between single nucleotide polymorphisms (SNPs) in four genes (*AKR1C3-Gln5His*, *NQO1-Pro187Ser*, *MnSOD-Val16Ala* and *OGG1-Ser326Cys*) that play a role in the generation, prevention or repair of oxidative damage and lung cancer risk in a population-based, case-control study of 119 cases and 113 controls in Xuan Wei, China. The *AKR1C3-Gln/Gln* genotype was associated with a 1.84-fold [95% confidence interval (CI) = 0.98–3.45] increased risk and the combined *OGG1-Cys/Cys* and *Ser/Cys* genotypes were associated with a 1.93-fold (95% CI = 1.12–3.34) increased risk of lung cancer. Subgroup analysis revealed that the effects were particularly elevated among women who had relatively high cumulative exposure to smoky coal. SNPs in *MnSOD* and *NQO1* were not associated with lung cancer risk. These results suggest that SNPs in the oxidative stress related-genes *AKR1C3* and *OGG1* may play a role in the pathogenesis of lung cancer in this population, particularly among heavily exposed women. However, due to the small sample size, additional studies are needed to evaluate these associations within Xuan Wei and other populations with substantial exposure to PAHs.

Abbreviations: AKR, aldo-keto reductases; PAH, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; SNP, single nucleotide polymorphisms.

Introduction

Residents in Xuan Wei, China are exposed to exceptionally high levels of polycyclic aromatic hydrocarbons (PAHs) from conception through adulthood via the smoky coal they burn for heating and cooking (1–5). Non-smoking women can inhale 10-times more PAH than a 20 cigarette/day active smoker, and air concentrations can approach levels experienced by workers on the top-side of coke ovens (5). Lung cancer rates in Xuan Wei County, China are among the highest in China and are similar for men and women (27.7 and 25.3 per 100 000 for men and women in the county, respectively) (5), despite the fact that almost all women are non-smokers. The lung cancer mortality rate in the three Xuan Wei communes where the most common smoky coal used has a particularly high polycyclic aromatic hydrocarbon (PAH) content was 118.0 and 125.6 per 100 000, for men and women, respectively, adjusted to the Chinese 1964 population, and 186.8 and 193.4 per 100 000 when adjusted to the US 1970 population.

Previous studies in this population have provided strong support that the lung cancer excess here is caused by smoky coal use among both men and women with only a modest contribution made by tobacco use among men (1,3–6). As such, the population in Xuan Wei provides a relatively unique opportunity to study the pathogenesis of PAH-induced lung cancer that is not influenced by the large number of other carcinogenic constituents of tobacco.

PAHs are activated to genotoxic intermediates through at least three primary pathways (Figure 1), all of which can lead to the production of G to T transversions (7). One of these pathways involves dihydrodiol dehydrogenases, which are members of the aldo-keto reductase (AKR) superfamily, and this pathway has been shown experimentally to produce PAH metabolites that form DNA adducts or reactive oxygen species (ROS) leading to oxidative DNA damage, such as 8-hydroxy-2′-deoxyguanosine (8-oxo-dG) (7). This superfamily includes *AKR1A1*, *AKR1C1*, *AKR1C2*, *AKR1C3* and *AKR1C4* (7–10). AKR1C3 is a particularly important enzyme in metabolizing potent *trans*-dihydrodiols containing more than two rings (7). Auto-oxidation of the intermediate catechols and/or subsequent redox cycling of the quinones can generate ROS that cause oxidative DNA damage (7,11,12).

Although single nucleotide polymorphisms (SNPs) in several genes in this pathway could influence the risk for lung cancer associated with cigarette smoke (13–15), which contains PAHs, SNPs in the *AKR* gene itself have not yet been examined for their influence on risk for cancer in an epidemiology study. Other metabolic enzymes that play a role in this process include *NQO1*, which reduces quinones back to catechols (16) and *MnSOD*, which can detoxify ROS in some
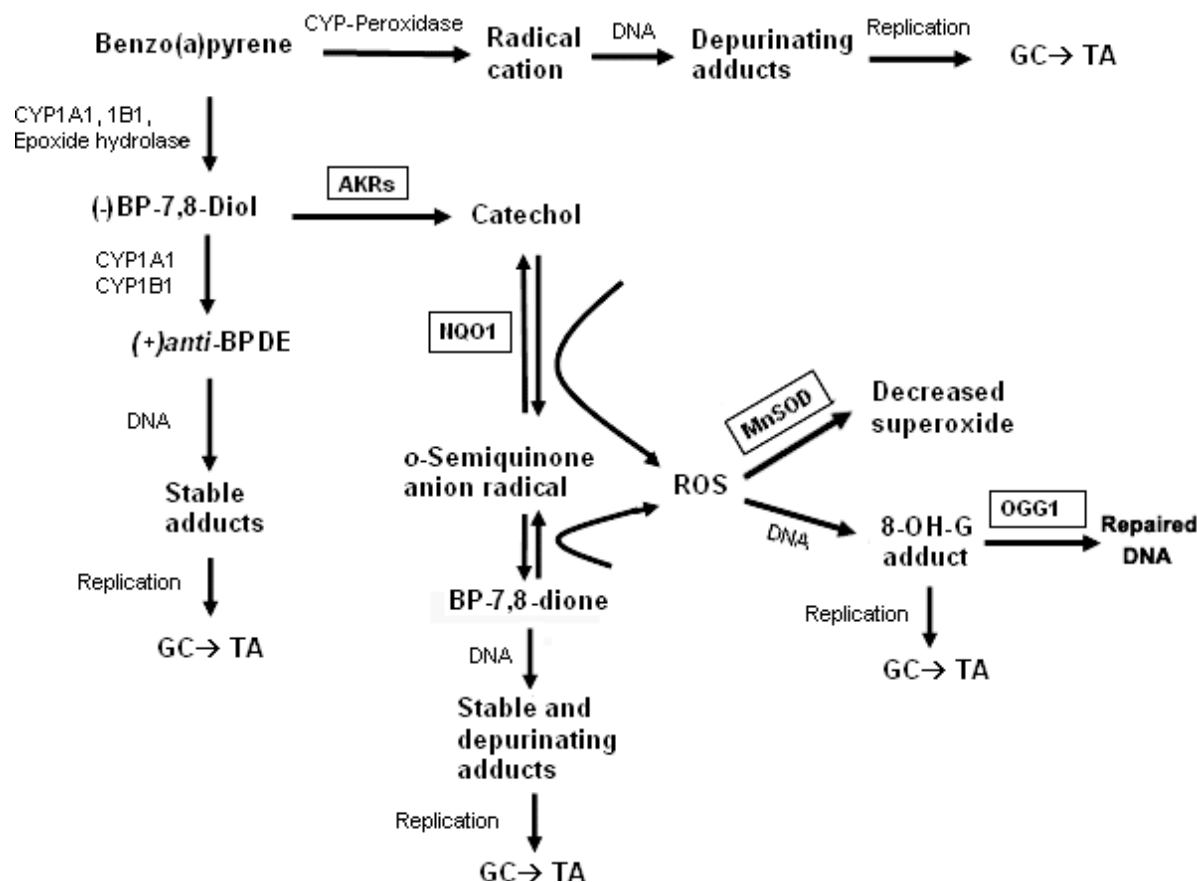
Fig. 1. Pathways for activation of benzo(a)pyrene, modified from Palackal *et al.* (1). The four genes whose SNPs were examined here are noted in boxes and span the entire pathway for the oxidative metabolism of PAHs.

instances (17). Multiple DNA repair mechanisms exist to avoid the effects of ROS and maintain genomic integrity (18,19). The most important DNA repair pathway of oxidative damage is base-excision repair (20) and includes the enzyme OGG1 (20–22).

Here we have evaluated the influence of a novel non-synonymous SNP in *AKR1C3-Gln5His* on lung cancer risk, as well as SNPs in other genes in this pathway (*NQO1*, *SOD2* and *OGG1*), in a population-based, case-control study among residents of Xuan Wei County, China who have an extremely high PAH exposure due to the use of smoky coal in poorly vented homes (5). The results are considered in the context of previous reports on hot spot sites of PAH stable adducts in the *TP53* gene in lung tumors from tobacco smokers (23) and hot spots of G to T transversions in lung tumors from non-smoking women exposed to smoky coal in Xuan Wei (24).

Materials and methods

This population-based, case-control study was described in detail elsewhere (3,25). Briefly, this study, which was carried out during the period from March 1995 to March 1996, contained a total of 122 newly diagnosed lung cancer cases. One control was selected for each lung cancer case, matching on sex, age ($\pm$2 years), village and type of fuel used currently for cooking and heating at home. As we described previously (3), the criteria for inclusion as a lung cancer case for this study were a histology or cytology confirmed (105 cases, 86.1%) or clinically diagnosed cases who died within a 1-year period (17 cases, 13.9%) (3). Information about demographic characteristics, lifetime consumption of different types of coal, tobacco smoking, family history of lung cancer and personal medical history were obtained from a standardized closed-question form of questionnaire. DNA was extracted from sputum samples

using phenol–chloroform extraction (26). Genotyping was carried out by real-time PCR on an ABI 7900HT sequence detection system as described on the SNP500 website (27). Of the 122 cases and 122 controls, DNA was successfully extracted from 119 cases and 113 controls. For human subject protection, this study was conducted according to the recommendations of the World Medical Association Declaration of Helsinki. The research protocol was approved by a US EPA Human Subjects Research Review Official for international research projects, and informed consent was obtained from all subjects in this study.

Genotype data were analyzed with the most common genotype as the referent, with the exception of *AKR1C3*, in which it is plausible that the most common variant could be associated with the greatest production of ROS. Genotype data for the four SNPs were not significantly correlated with each other or with pack-years of smoking, smoky coal use or the *GSTM1* null genotype, which we have shown previously was associated with increased lung cancer risk in this population (3). The test for Hardy–Weinberg equilibrium among the controls was conducted using observed genotype frequencies and a χ^2 test with one degree of freedom. Unmatched unconditional logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for age, sex, and pack-years of tobacco use, to test the association between lung cancer risk and genotypes and other risk factors so that all the genotype data could be used. Crude ORs were essentially identical to adjusted ORs, with the exception of the *AKR1C3* association among women with heavy smoky coal use, which is presented in a footnote in Table III. Further adjustment for village and fuel type produced similar results in almost all models; however, in a few instances the ORs were substantially inflated, so these variables were not adjusted for in the analyses presented here. Data were analyzed using Statistical Analysis Software, version 8.02 (SAS Institute, 1996).

The OR for smoky coal use was estimated for the mean amount of lifetime cumulative tons of smoky coal, based on the distribution in the controls. In addition, smoky coal use was modeled as a linear relationship between the total amount of smoky coal used and the log odds of lung cancer. Gene–environment interactions were tested on a multiplicative scale by adding product terms into a logistic regression model.

Results

Table I shows the distribution of characteristics for both cases and controls. Among cases and controls, age, sex and type of fuel source were comparable. Approximately 34% of the cases and controls were female. Ethnicity, education level, household income and dwelling type were comparable for cases and controls (not shown). About 93% of the males were tobacco smokers, whereas only one female smoked. Among men, smoking for >25 pack-years was associated with a 1.69-fold (95% CI = 0.82–3.49) increased risk of lung cancer. This relatively weak smoking effect was consistent with previous studies in Xuan Wei (1,3,4,6) and may have been due to the use of water pipes for smoking. Cases were more likely to be heavy smoky coal users than controls. Compared with subjects who used <130 tons of smoky coal during their lifetime, heavy smoky coal users (those who used >130 tons) had a 2.27-fold (95% CI = 1.25–4.10) increased risk of lung cancer. Among women, the risk was 6.11 (95% CI = 1.92–19.43) for heavy smoky coal use, and among men the risk was 1.49 (95% CI = 0.73–3.04).

Table I. Distribution of subject characteristics in lung cancer patients and controls

	Case n (%)	Control n (%)	P value[a]
Age			
<55	50 (42)	46 (41)	
≥55	69 (58)	67 (59)	0.84
Sex			
Male	78 (66)	73 (65)	
Female	41 (34)	40 (35)	0.88
Smoking[b]			
No	5 (6)	5 (7)	
Yes	73 (94)	68 (93)	0.91
Smoky coal use			
<130	50 (42)	67 (59)	
≥130	69 (58)	46 (41)	0.008

[a]P based on χ^2 test.
[b]Males only.

Table II presents the association of genotypes with lung cancer risk. All genotype distributions were in Hardy–Weinberg equilibrium. We observed an increased lung cancer risk for the *AKR1C3* and *OGG1* genotypes, but not for the *NQO1* or *SOD2* genotypes (Table II). We further stratified the analysis of each genotype on lung cancer risk by both sex and smoky coal use in an attempt to separate the effects of each (Table III). There was a consistent pattern in that the impact of both the *AKR1C3* and *OGG1* genotypes on lung cancer risk was particularly pronounced among women exposed to >130 tons of smoky coal. The test for interaction between sex, smoky coal use as a dichotomous or continuous variable and each genotype was not, however, significant. Effects were similar among smokers with >25 pack-years of tobacco use and smokers with <25 pack-years, and tests for interaction with pack-year category or with pack-years as a continuous variable were not significant.

Discussion

The AKR superfamily, which includes *AKR1C3*, has been shown to oxidize PAHs to catechol, which can then produce a-dione that can form DNA adducts and/or ROS, which can cause oxidative damage to DNA (Figure 1). The *AKR1C3-Gln5His* SNP produces a glutamine-to-histidine amino acid change, but the impact on enzymatic activity and whether it is in linkage disequilibrium with other potentially functional variants is not known. Our results are consistent with the hypothesis that alterations in the relative contribution of AKR1C3 to the metabolism of PAHs could impact lung cancer risk. To our knowledge, this is the first report of a molecular epidemiological study that has suggested that genetic variation in *AKR1C3* could be associated with risk for lung cancer. Comprehensive evaluation of additional SNPs in this gene are needed to estimate the importance of common haplotypes; moreover, additional studies of other AKR superfamily members are needed to characterize further the role of perturbations of these genes in the pathogenesis of PAH-associated lung cancer.

Table II. *AKR1C3-Gln5His*, *NQO1-Pro187Ser*, *MnSOD2-Val16Ala* and *OGG1-Ser326Cys* polymorphisms and lung cancer risk

Genotype	dbSNP ID	Case n (%)	Control n (%)	OR[a] (95% CI)	P value
AKR1C3	rs12529				
His/His		1 (0.86)	1 (0.89)	–	
His/Gln		21 (18.10)	32 (28.57)	–	
His/His + His/Gln		22 (18.97)	33 (29.46)	1.0	
Gln/Gln		94 (81.03)	79 (70.54)	1.84 (0.98–3.45)	0.06
NQO1	rs1800566				
Pro/Pro		37 (31.09)	32 (29.36)	1.0	
Pro/Ser		57 (47.90)	54 (49.54)	0.90 (0.49–1.66)	0.75
Ser/Ser		25 (21.01)	23 (21.10)	0.90 (0.43–1.90)	0.79
Pro/Ser + Ser/Ser		82 (68.91)	77 (70.64)	0.91 (0.51–1.62)	0.73
SOD2	rs1799725				
Val/Val		93 (78.15)	81 (72.32)	1.0	
Val/Ala		23 (19.33)	30 (26.79)	0.67 (0.36–1.25)	0.20
Ala/Ala		3 (2.52)	1 (0.89)	–	
Val/Ala + Ala/Ala		26 (21.85)	31 (27.68)	0.73 (0.40–1.33)	0.30
OGG1	rs1052133				
Ser/Ser		37 (31.36)	51 (46.79)	1.0	
Ser/Cys		61 (51.69)	43 (39.45)	1.96 (1.10–3.57)	0.02
Cys/Cys		20 (16.95)	15 (13.76)	1.85 (0.83–4.11)	0.13
Ser/Cys + Cys/Cys		81 (68.64)	58 (53.21)	1.93 (1.12–3.34)	0.02

[a]ORs and 95% CIs obtained by logistic regression analysis adjusted for age, sex, pack-years of smoking.

Table III. *AKR1C3-Gln5His* and *OGG1-Ser326Cys* polymorphisms and lung cancer risk stratified by smoky coal use and sex

Genotype	Smoky coal use without a chimney (tons)							
	<130 tons				≥130 tons			
	Case *n* (%)	Control *n* (%)	OR[a] (95%CI)	*P* value	Case *n* (%)	Control *n* (%)	OR[a] (95% CI)	*P* value
Female								
AKR1C3								
His/His + His/Gln	4 (33.33)	8 (33.33)	1.0		5 (17.24)	10 (66.67)	1.0	
Gln/Gln	8 (66.67)	16 (66.67)	1.00 (0.19–5.80)[b]	1.00	24 (82.76)	5 (33.33)	12.93 (2.20–107.82)[bc]	0.0018
OGG1								
Ser/Ser	5 (41.67)	11 (47.83)			4 (13.79)	8 (53.33)		
Ser/Cys + Cys/Cys	7 (58.33)	12 (52.17)	1.29 (0.31–5.28)	0.73	25 (86.21)	7 (46.67)	5.67 (1.07–34.16)[b]	0.04
Male								
AKR1C3								
His/His + His/Gln	8 (21.62)	11 (25.58)	1.0		5 (13.16)	4 (13.33)	1.0	
Gln/Gln	29 (78.38)	32 (74.42)	1.49 (0.50–4.43)	0.48	33 (86.84)	26 (86.67)	0.94 (0.17–4.95)[b]	1.00
OGG1								
Ser/Ser	15 (40.54)	18 (43.90)			13 (32.50)	14 (46.67)		
Ser/Cys + Cys/Cys	22 (59.46)	23 (56.10)	1.14 (0.45–2.90)	0.78	27 (67.50)	16 (53.33)	2.04 (0.74–5.60)	0.17

[a]ORs and 95% CIs obtained by logistic regression analysis adjusted for age, pack-year of smoking.
[b]Exact test.
[c]Crude OR = 9.60 (2.27–40.62), *P* = 0.0021.

Consistent with the role of the AKR pathway in non-smokers with heavy smoky coal use is our finding that subjects with *OGG1-Cys/Cys* and *Ser/Cys* alleles were also at increased risk for lung cancer. Some (28,29) but not all (30) studies have provided evidence that this variant is associated with lung cancer risk, and there is evidence that it results in decreased DNA repair activity (13,31). *OGG1* is part of the base-excision DNA repair system and removes oxidatively damaged bases, such as 8-oxo-G. Such damage would be expected as a consequence of metabolism of PAHs through the AKR pathway. In this regard, a recent study in mammalian cells found that *OGG1* suppressed G to T transversions due to 8-oxo-G, but not those due to benzo[*a*]pyrene diol-epoxide adducts (32). Thus, we have considered the influence of this pathway on the mutation spectrum in smoky coal-associated lung tumors (24).

We identified previously three hot spots for mutations (G to T transversions) in the *TP53* gene in lung tumors of non-smoking women in Xuan Wei exposed to smoky coal emissions (24). Two of these mutational hot spots are at codons 154 and 273, which are also hot spots for PAH adduction (stable adducts) (23). We therefore postulated that stable PAH adducts, most likely produced through the CYP pathway (Figure 1) could be responsible for the G to T transversion at each site (24). However, the third mutational hot spot is at codon 249, which is not a hot spot for stable PAH adduction (23,24). Our present result leads us to suggest that G to T transversions at this site (codon 249) may be due to oxidative DNA damage resulting from the metabolism of PAHs in the smoky coal emissions via the AKR pathway. Such a pathway could produce unstable (depurinating) adducts via the production of the PAH-quinone, resulting in apurinic sites, or ROS that could result in oxidative damage, such as 8-oxo-G. Either type of damage, if not repaired, can be processed by the cell into G to T transversions (7,8,10). Thus, we propose that the mutation spectrum in smoky coal-associated tumors reflects PAH damage due both to stable adducts as well as to unstable adducts and/or oxidative base damage due to ROS production.

The *Pro187Ser* variant of *NQO1*, which is an oxidoreductase enzyme that plays a role in the reactions described in Figure 1, results in decreased enzyme activity (15,16); however, it was not associated with lung cancer risk in our study. Overall, there have been both positive and null reports of its association with lung cancer (15,16,33). Similarly, the *Val16Ala* SNP in *SOD2*, which has been predicted to result in higher enzymatic activity (34), was not associated with increased risk in this study. There has been a positive association of this SNP with lung cancer risk in a Caucasian population but not in a Taiwanese population (14,33).

Our study has several strengths. It is a population-based, case-control study with high participation rates in a unique semi-mountainous rural area of China where environmental PAH exposure is 10-times higher than that received by a 1-pack/day smoker and can approach concentrations experienced by coke-oven workers. This contrasts with almost all other molecular epidemiology studies of lung cancer reported previously in the literature. Further, our series of lung cancer cases is unique in that smoky coal exposure appears as the primary etiological factor, in contrast to tobacco in almost all other populations studied. This is particularly so for women in Xuan Wei, whose lung cancer incidence is the highest in China (5). As a consequence, this study population provides a unique group to explore the influence of variation in key genes that mediate the effects of PAHs on lung cancer risk. Finally, the pattern of particularly elevated genetic risks among women who were heavy smoky coal users is consistent with the effects of the *GSTM1* null genotype on lung cancer risk in this population (unpublished data).

The primary limitation of our study is its small sample size and consequently low power. As pointed out recently by Wacholder *et al.* (35), a statistically significant association in a small study has an increased likelihood of being a false positive finding, particularly if the risk factor has a low prior probability. In addition, some investigators are concerned about multiple comparisons increasing the probability of detecting statistically significant yet false positive findings. We evaluated the overall effects of four SNPs and carried out a number of subgroup analyses. The observed low *P*-value of the association between the *AKR1C3-Gln/Gln* genotype and lung cancer in heavily exposed women would meet the standard of statistical significance that would be required

by procedures that attempt to correct for multiple comparisons. Clearly, however, the findings from this study need to be considered preliminary. A new, substantially larger case-control study of lung cancer among non-smoking women is being planned among this relatively unique population and will provide an opportunity to replicate and extend these findings. Finally, more extensive analysis of *AKR1C3* is needed to comprehensively assess the contribution of genetic variation across the gene (36) to lung cancer risk and to study the impact of genetic variation on enzyme activity.

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